RESEARCH NOTE

Bactec 9240 blood culture system: to preincubate at 35 °C or not?

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ABSTRACT

Bactec Plus blood culture bottles were preincubated at 35°C or at room temperature before entry into the Bactec 9240 instrument to determine the influence of preincubation temperature and time. Of 463 positive blood culture sets, 956 bottles were positive, of which the instrument detected 92.1%. Of 76 positive bottles undetected by the instrument, 68 were preincubated at 35°C and eight at room temperature. The median entry delay and instrument detection times were 17.9 and 7.2 h for preincubated bottles, and 16.4 and 13.4 h for bottles held at room temperature. Short entry delay and inspection before entry into the instrument are necessary if preincubation at 35°C is used.

Keywords Bactec system, blood culture, preincubation of blood cultures

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Rapid and maximal detection of bacteraemia is important, requiring emphasis on optimal blood culture system technology and utilisation [1,2]. Concerns arose in our laboratory regarding the performance of the Bactec 9240 system (Becton Dickinson, Cockeysville, MD, USA) when seven of 69 bottles were negative by the Bactec instrument, but positive on terminal subculture. Audit revealed that the manufacturer's recommendations [3] were unclear concerning the advisability of preincubation at 35°C before entry into the instrument. At our hospitals, bottles were preincubated [4], sometimes for >20 h. Therefore, this study aimed to determine the influence of preincubation time and temperature on performance.

Blood culture sets (except those received on Sundays) were enrolled consecutively from July 2001 to March 2002. A set consisted (in 96.5% of cases) of two Bactec Plus Aerobic/F bottles and one Anaerobic/F bottle. Blood culture sets were preincubated at 35°C at two hospitals (Køge and Rønne Hospitals) and stored at room temperature at Roskilde Hospital until once-daily transport to the laboratory. Roskilde and Køge Hospitals, which are community hospitals serving the county of Roskilde, contributed 85% of the blood cultures, while Rønne Hospital contributed 15%. Hospital services and patient populations were comparable between the two groups.

Bottles were inspected upon arrival, and visually positive bottles were subcultured. In addition, one aerobic bottle from visually negative sets was subcultured ('initial subculturing'). All bottles were incubated in the Bactec 9240 instrument for 5 days, or until detected as positive (software version 4.01B). Bottles not detected as positive after 5 days were subcultured ('terminal subculturing'). Positive bottles were bottles yielding growth by any of these methods. 'Entry delay' was defined as the time between blood sampling and bottle entry into the instrument, while 'instrument detection time' was defined as the time between entry into the instrument and detection. Fisher's exact test was used for data comparisons, and a Kaplan–Meier plot was used to compare instrument detection times.

Of 4006 blood culture sets entered, 463 sets (11.6%) were positive, with 956 bottles yielding 1010 organisms. Of these 956 bottles, 511 (53.5%) had been stored at room temperature and 445 (46.5%) had been preincubated at 35°C. The instrument detected 880 (92.1%) positive bottles, of which 503 (98.4%) had been stored at room temperature and 377 (84.7%) had been preincubated at 35°C ($p < 0.0001$). On arrival, 97 bottles were visually positive and yielded growth. Of these, 82 had been preincubated, and 40 were not detected by the instrument. All 15 visually positive bottles stored at room temperature were detected by the instrument. Initial and terminal subculturing revealed 36 bottles not detected by

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other methods. Thus, preincubation at 35°C resulted in a significantly higher false-negative detection rate compared to storage at room temperature (p 0.0002).

Of 1010 organisms recovered from positive bottles (Table 1), 929 (92.0%) were instrument-detected. Table 2 shows the 81 organisms, isolated from 76 bottles, that were not detected by the instrument. Of 413 Enterobacteriaceae and 485 Gram-positive cocci, 18 (4.4%) and 45 (9.3%), respectively, were not detected by the instrument. Streptococci and enterococci were undetected in 17% of cases; 90% of these isolates were positive on visual examination, and half of these sets had an entry delay of < 20 h.

Initial and terminal subculture of otherwise negative bottles yielded 37 organisms from 36 bottles (Table 2). Only six organisms found on initial subculture were not detected by the instrument, and five of these were recovered on terminal subculture. Thus, terminal subculture yielded 36 organisms from 35 bottles that were not detected by either visual inspection or the instrument. Twenty-six of these 36 organisms were detected by other methods from the same blood culture set. The remaining ten organisms comprised six coagulase-negative staphylococci, presumably contaminants, and one each of *Candida albicans*, *Pseudomonas aeruginosa* and *Candida glabrata* found on terminal subculture, plus one *Staphylococcus aureus* found on initial subculture. Thus, the gain from subculturing was minimal.

The median entry delay was available for 358 positive sets and was 17.4 h, but was 1.5 h less after storage at room temperature than for those that were preincubated. The median entry delay for visually positive sets was 19.2 h, but was 3.5 h longer after storage at room temperature than for those that were preincubated. The overall median instrument detection time for 432/442 sets detected by the instrument was 11.5 h, but was less, with a median of 7.2 h, for the bottles preincubated at 35°C (p 0.049).

No recommendations were found in the Danish instrument manual [5] regarding inspection or storage of bottles, while the English-language manual [6] contains a recommendation for inspection of bottles upon arrival, but no recommendations concerning storage temperature or time.

Table 1. Identification of 1010 microorganisms isolated from 956 positive blood culture bottles

Microorganism	No. of isolates		
	Total recovered	Bactec positive	Bactec negative
Gram-negative aerobic bacilli (*n* = 450)			
Citrobacter spp.	6	6	0
Enterobacter spp.	14	14	0
Escherichia coli	296	286	10
Klebsiella spp.	66	63	3
Morganella morganii	6	2	4
Pantoea agglomerans/Erwinia herbicola	4	3	1
Proteus spp.	12	12	0
Salmonella spp.	5	5	0
Serratia marcescens	4	4	0
Aeromonas spp.	3	3	0
Haemophilus influenzae	3	3	0
Acinetobacter spp.	11	6	5
Pseudomonas aeruginosa	15	10	5
Other '*Pseudomonas*' spp.	5	4	1
Gram-negative diplococci (*n* = 5)			
Neisseria meningitidis	4	4	0
Moraxella catarrhalis	1	1	0
Gram-positive cocci (*n* = 485)			
Staphylococcus aureus	103	96	7
Coagulase-negative staphylococci	215	206	9
Aerococcus urinae	3	3	0
β-Haemolytic streptococci	29	22	7
Streptococcus pneumoniae	62	47	15
Other streptococci	35	35	0
Enterococcus spp.	38	31	7
Gram-positive bacilli (n = 19)			
Corynebacterium spp.	13	13	0
Other Gram-positive bacilli	6	6	0
Anaerobes (*n* = 22)	22	22	ND
Yeasts (*n* = 29)			
Candida albicans	17	13	4
Candida glabrata	4	1	3
Other yeasts	8	8	0
Total	1010	929	81

ND, not done; anaerobic subculturing was not performed.

Table 2. Identification of 81 microorganisms isolated from 76 positive blood culture bottles that were not detected by the Bactec instrument

Microorganism	No. of isolates				
	Total Bactec negative	Visually positive		Visually negative, but subculture-positive[a]	
		Aerobic bottles	Anaerobic bottles	Aerobic bottles	Anaerobic bottles
Gram-negative aerobic bacilli					
Escherichia coli	10	5	0	5	0
Klebsiella spp.	3	1	1	1	0
Morganella morganii	4	2	0	2	0
Pantoea agglomerans	1	0	0	1	0
Acinetobacter spp.	5	2	1	1	1
Pseudomonas aeruginosa	5	1	0	0	4[b]
Other '*Pseudomonas*' spp.	1	0	0	0	1
Gram-positive cocci					
Staphylococcus aureus	7	2	2	1[b]	2
Coagulase-negative staphylococci	9	0	1	4[c]	4[c]
β-Haemolytic streptococci	7	4	3	0	0
Streptococcus pneumoniae	15	11	4	0	0
Enterococcus spp.	7	2	2	3	0
Yeasts					
Candida albicans	4	0	0	1[b]	3
Candida glabrata	3	0	0	1	2[b]
Total	81	30	14	20	17

[a]Implies culture-positive on initial or terminal subculture.
[b]Includes one case where the bottle was the only positive bottle of a set.
[c]Includes three cases where the bottle was the only positive bottle of a set.

According to Shigei *et al.* [7], the manufacturer recommends that the duration of preincubation should not exceed 8 h at 35°C or 16 h at room temperature. Chapin and Lauderdale [8] state that the manufacturer recommends that bottles can be held at 35°C for up to 20 h. The manufacturer advised (personal communication) that the duration of preincubation at 35°C should not exceed 20 h, and that room temperature should be used for an entry delay of >20 h. The data for this recommendation were obtained from simulated blood cultures [8]. Our laboratory often received blood culture sets after an entry delay of >20 h. Despite inspection of preincubated bottles upon arrival at the laboratory, Ziegler *et al.* [9] found that an entry delay of >12 h resulted in a significantly lower detection rate than in the absence of delay. With simulated blood cultures, Chapin and Lauderdale [8] reported a 2.1% loss of detection by Bactec for selected species preincubated at 35°C for 24 h, and a loss of 5.3% after preincubation for 36 h.

Our finding that Bactec 9240 detected only 92.1% of the positive bottles suggests the advisability of short entry delays and visual inspection of bottles if preincubation at 35°C is used. The clinical gain from shorter instrument detection time after preincubation at 35°C must be weighed against the loss of microbiological data as a result of false-negative bottles in the instrument. The fact that, in contrast to preincubated bottles, all visually positive bottles stored at room temperature were detected by the instrument suggests that some of the organisms in the preincubated group were beyond the logarithmic phase of growth. The latest Bactec 9240 software (v. 4.01B) uses growth and kinetic algorithms without threshold algorithms. A decrease in the sensitivity of this system was reported [8] for bottles with delayed entry when the Delayed Vial Entry application (v. 3.06B software with threshold algorithms) was compared with a newer version (v. 3.40H) without threshold algorithms. Reintroduction of threshold algorithms into the system, together with implementation of shorter entry delays, might further improve the performance of this widely used blood culture system.

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